****

**Lab One: Spikes for all!**

**A Beginner’s Guide to the SpikerBox**

**Overview**

**In this lab you will:**

1. learn how to prepare a cockroach leg for electrophysiological readings;
2. observe and record action potentials generated in a cockroach leg using a SpikerBox and a computer; and
3. investigate how different types of stimulations affect the generation of action potentials in a cockroach leg preparation.

**Objectives**

**Before doing this lab you should understand:**

* the purpose and shape of an action potential
* the basics of electrophysiology
* how measuring action potentials from either inside or outside of a cell affects the data collected
* the basic anatomy of the cockroach leg and nervous system

**After doing this lab you should be able to:**

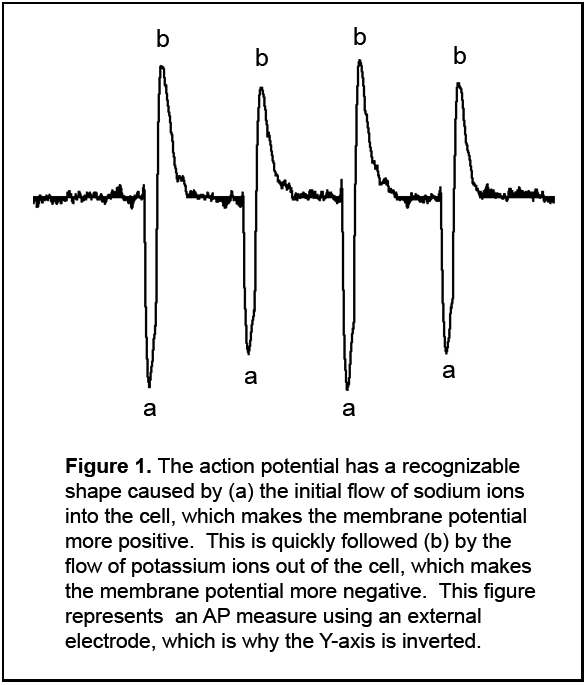
* distinguish between intra- and extracellular recordings
* perform basic analysis of electrophysiology data
* design basic electrophysiology experiments to study the nervous system of insects
* understand how sensations enter into the nervous system
* explain how an organism can differentiate between multiple types of sensory stimulations
* describe the concept of rate coding

**EQUIPMENT**

* SpikerBox with audio cable adapter
* cockroach
* ice water or access to a freezer
* dissection scissors
* toothpicks
* drinking straw

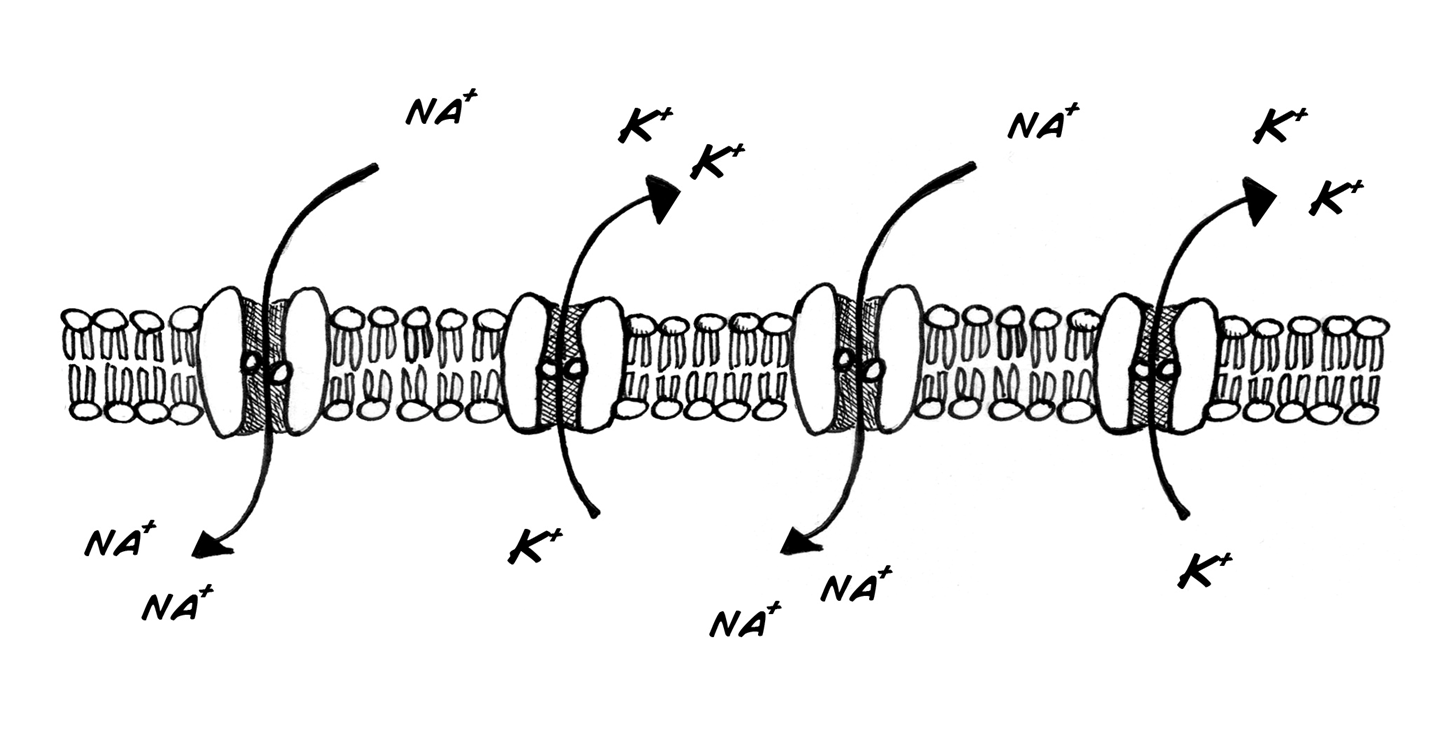
**Introduction**

**Action Potentials:** An action potential (AP) is an electrical event in a small section of cell membrane that acts as fast moving, long-range signal. The AP, once initiated, will spread down the membrane of a cell, thus allowing a cell to communicate with astonishing speed (80 m/s). Neurons can send APs, or spikes, in specific patterns down their axon to convey information at a synapse, the point at which one neuron communicates with another through chemical signaling.

The main feature of an AP is the distinctive shape (Figure 1). This graph shows the charge across a section of membrane over a very short period of time. When a cell is at rest, or not sending an AP, it will be at resting potential. Resting potential is the resulting voltage established by a cell concentrating various ions (Na+, K+, Cl-) on different sides of its membrane. This uneven distribution of ions creates a negative charge on the inside of the membrane.

If a neuron is stimulated enough it will generate an AP. During the AP, the membrane has a fast rise and fall in potential caused by the movement of Na and K across the membrane, respectively. The movement of these ions can be measured using electrophysiology.

**Synapses:** Synapses are the point of communication between two neurons. While an AP is an electrical signal that allows a neuron to communicate along the length of its axon, it is not a signal that can jump from one cell to the next. This requires a neuron to convert the electrical signal to a chemical one in order to successfully communicate. The location of this event is the synapse.

In a synapse, a neuron sending the signal, the presynaptic cell, will stimulate a receiving neuron, the postsynaptic cell, by placing a chemical signal into the synapse. Called a neurotransmitter, this chemical signal will either excite or inhibit the postsynaptic cell. You may already know several neurotransmitters. Several well-known ones secreted by the presynaptic cell are glutamate, GABA, dopamine, serotonin, and acetylcholine (ACh). The postsynaptic cell has the specific receptor for the neurotransmitter, which causes changes in electrical excitability once bound.

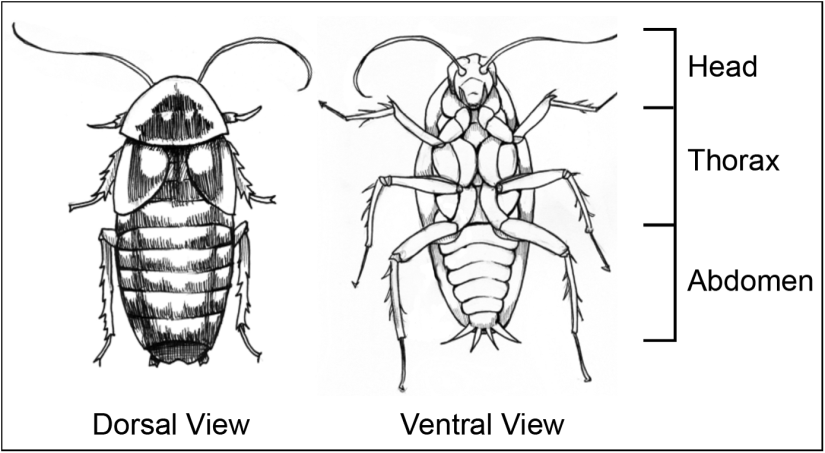
View of Neural Membrane showing flux of ions through channels.

**Electrophysiology:** Electrophysiology, the study of the electrical properties of cells, allows researchers to study how neurons communicate with each other and form complex neural networks. In particular, scientists and physicians use electrophysiology to measure the properties of APs in living neurons. Two of the most common approaches used to study APs are to record from either an intracellular or extracellular perspective.

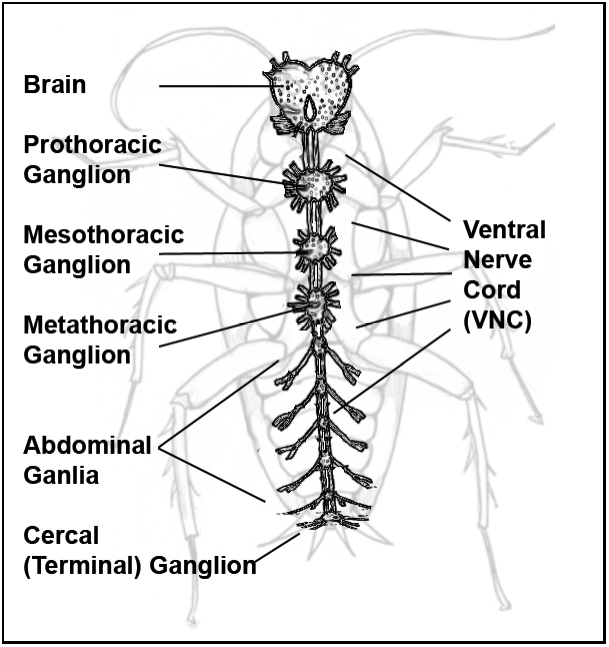
Intracellular recordings allow someone to measure either the voltage (charge) or current (movement of ions) across the membrane of a single cell. This technique requires the insertion of a recording electrode, housed inside a hollow glass pipette, into the membrane of a neuron. A second electrode, the ground electrode, is placed extracellularly. The difference in electrical activity (voltage or current) between the intracellular recording and extracellular ground electrodes reflects the electrical activity of the neuron being studied. For instance, an AP measured using this technique will look like those described in textbooks, with an initial positive depolarization followed by a negative repolarization and overshoot. It is important to remember that intracellular recordings are measuring the change in electrical properties across the cell membrane from the perspective of being inside the cell. This technique can be difficult to perform as the neuron impaled with the glass pipette cannot be moved more than a few microns without damaging or even killing the cell.

Extracellular recording is a second electrophysiological technique that measures the voltage and current across the membranes of neurons. However, rather than having to insert an electrode into a single cell, extracellular recordings can be made by simply placing a recording electrode adjacent to a cell membrane. For these experiments, measurements of charge and movement of ions across cell membranes will appear to be inverted compared to intracellular recordings. This is because the recording electrode is measuring ions entering and leaving the extracellular space.

Another important way in which data collected from intra- or extracellular recordings differ is the amplitude of APs collected. APs collected from intracellular recordings are consistent in their amplitude during an experiment. This is because there is little difference in how APs look once they are initiated. However, the amplitude of APs collected from extracellular electrodes can be of varying sizes for several different reasons. The first is that extracellular recordings may be measuring electrical activity from multiple cells at one time. If multiple axons near an extracellular electrode are sending APs down their axons, the resulting data will be larger. Additionally, larger axons can also increase the amplitude of a measured APs in an extracellular setup. However, keep in mind that the AP recorded by an extracellular electrode will get smaller the farther away the axon is from the point of measure. The SpikerBox is used to perform extracellular recordings.

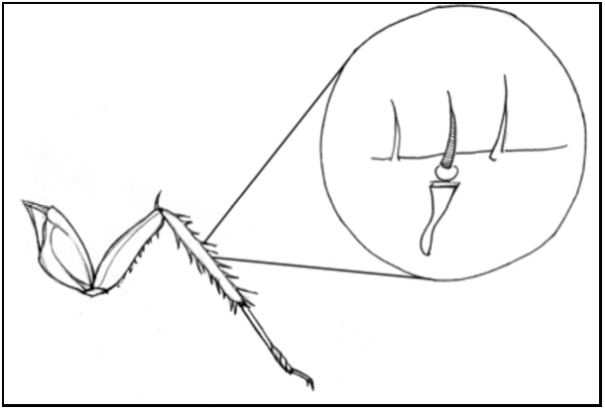
****

**Cockroach Anatomy and Senses:**

To observe the anatomy of a cockroach, first anesthetize one by placing it in a glass of ice water until it is no longer moving. The anatomy of the cockroach is exceptionally accessible to electrophysiological experimentation for a variety of reasons. First, from the dorsal, or top, view the cockroach has a distinctive prothorax (the section directly behind, and shielding the head) and wings that give the cockroach its distinctive armored look. When flipped on its back, the ventral aspect of the cockroach reveals the basic segmented body sections distinctive of insects: the head, thorax, abdomen, and legs. Identify the major delineations between these sections, and observe the segmentation inside these major regions.

For this lab, you will be removing a leg from the cockroach’s mesothorax, the last and largest leg closest to the abdomen. The major benefits of this approach are that the leg will grow back, and the cockroach nervous system provides wonderfully large APs that can be observed using the SpikerBox. Each segment of the cockroach contains a region of the Ventral Nerve Cord (VNC), a collection of neurons that send information to the muscles of the body, while receiving information from the sensory organs of the periphery. This information is relayed to and from the brain using action potentials and synapses. We look to measure these communications entering from the legs of the cockroach.

When observed up close, you can see how the cockroach leg is covered with large spines along the tibia and femur. Each spine has a neuron wrapped around it, which sends APs to the VNC and eventually the brain. The pattern and frequency of APs sent will allow the VNC to distinguish a strong external stimulus from a weak one. Which hair cells are being stimulated will determine where the cockroach perceives the stimulation is located.



**SETUP**

**Computer Setup**

Use of a computer for the basic aspects of this experiment is not required, as the SpikerBox is equipped with a small speaker. However, in order to perform some of the analysis described in this manual, a computer will be necessary.

There are several free options for capturing and analyzing data from your SpikerBox.

**Backyard Brains IPhone/iPad App:** Backyard Brains iPhone/iPad app, which can be downloaded via iTunes. This app can be found at the following link:

[**Backyard Brains iPhone/iPad app**](http://itunes.apple.com/us/app/backyard-brains/id367151200?mt=8)

To use this app, you will need to purchase or make an audio cable to link the SpikerBox to the iPhone.

**Audacity:** Audacity is a freeware program designed for editing and recording sounds. While not the original purpose of the software, Audacity is a perfect way to convert the amplified signal of the SpikerBox into visual and recordable data. Audacity runs on both Windows, Mac OS X, and Linux/Unix. The website for Audacity can found at the following link:

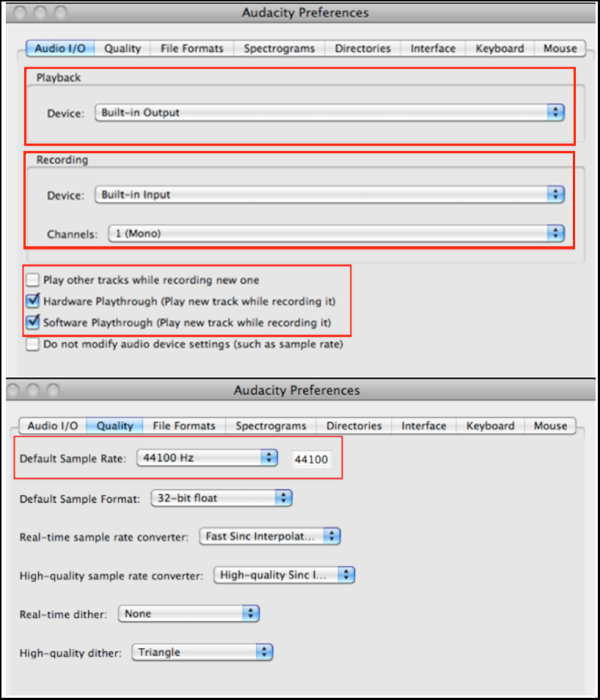
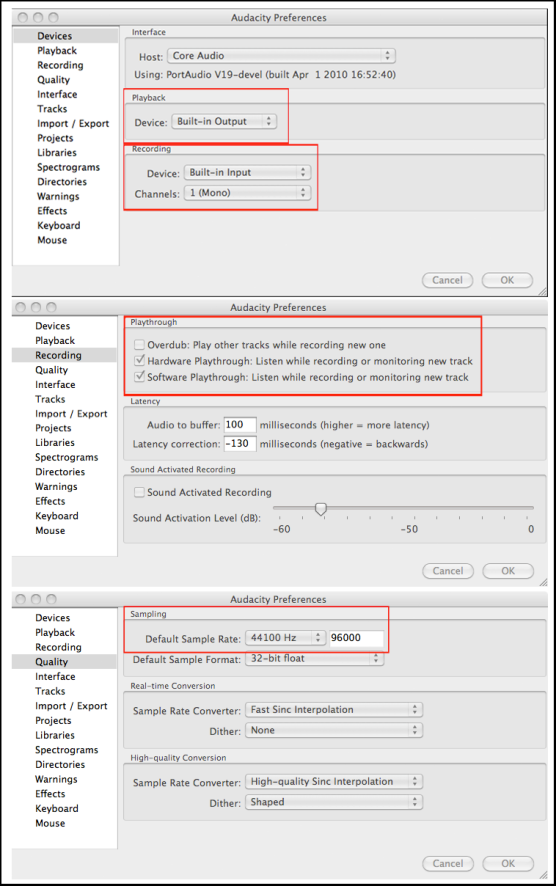
[**Audacity Homepage**](http://audacity.sourceforge.net/)

Once Audacity has been installed, refer to the following procedure:

**Audacity Setup**

1. If using a laptop, fully charge your battery and record from the SpikerBox while unplugged. When plugged in, the SpikerBox will amplify the current running through the electrical system of the building. The result will be an increase in electrical noise recorded by Audacity. Running off of battery power will decrease the amount of electrical noise.
2. If using a laptop, set the brightness of your screen to maximum. Similar to the effects of the surrounding electrical wiring on your recordings, a dimly lit LCD display will create a significant amount of noise that can be amplified by the SpikerBox.
3. Plug your SpikerBox into the Laptop with male-to-male audio cable. This is exactly the same as a headphone cable, except it has “male” connector on each end.
4. Open Audacity, go to **File 🡪 Preferences**. Note: Due to differences in Audacity between versions, there may be differences in how the **Preferences** window is organized. The primary settings you need to set up are the same, even if they look a little different on your screen.
5. Select the **Audio I/O** tab in the **Preferences** window. Some versions place this window in the **Devices** tab.
6. Select the **Built-in Input** as the **Recording Device** from the drop down menu.
7. Select **1 (Mono)** from the **Channels** drop down menu.
8. Select **Built-in Output** as the **Playback Device** from the drop down menu.
9. The following steps are found either in the **Audio I/O** or **Recording** tabs in the **Preferences** window. The following checkboxes are listed in order as they appear:
   * + **Uncheck** the box: Play other tracks while recording a new one.
     + **Check** the Box: Hardware Playthrough
     + **Check** the box: Software Playthrough
10. Select the tab labeled **Quality**. Set the **Default Sample Rate** to 44100 Hz (or 44,100 times per second).
11. Audacity should now be configured to record from your SpikerBox.

Below are examples of how your **Preferences** window should be configured. Keep in mind that your version of Audacity may look different, but the settings will be the same. Two examples are provided, with the important settings highlighted.



**PROCEDURE**

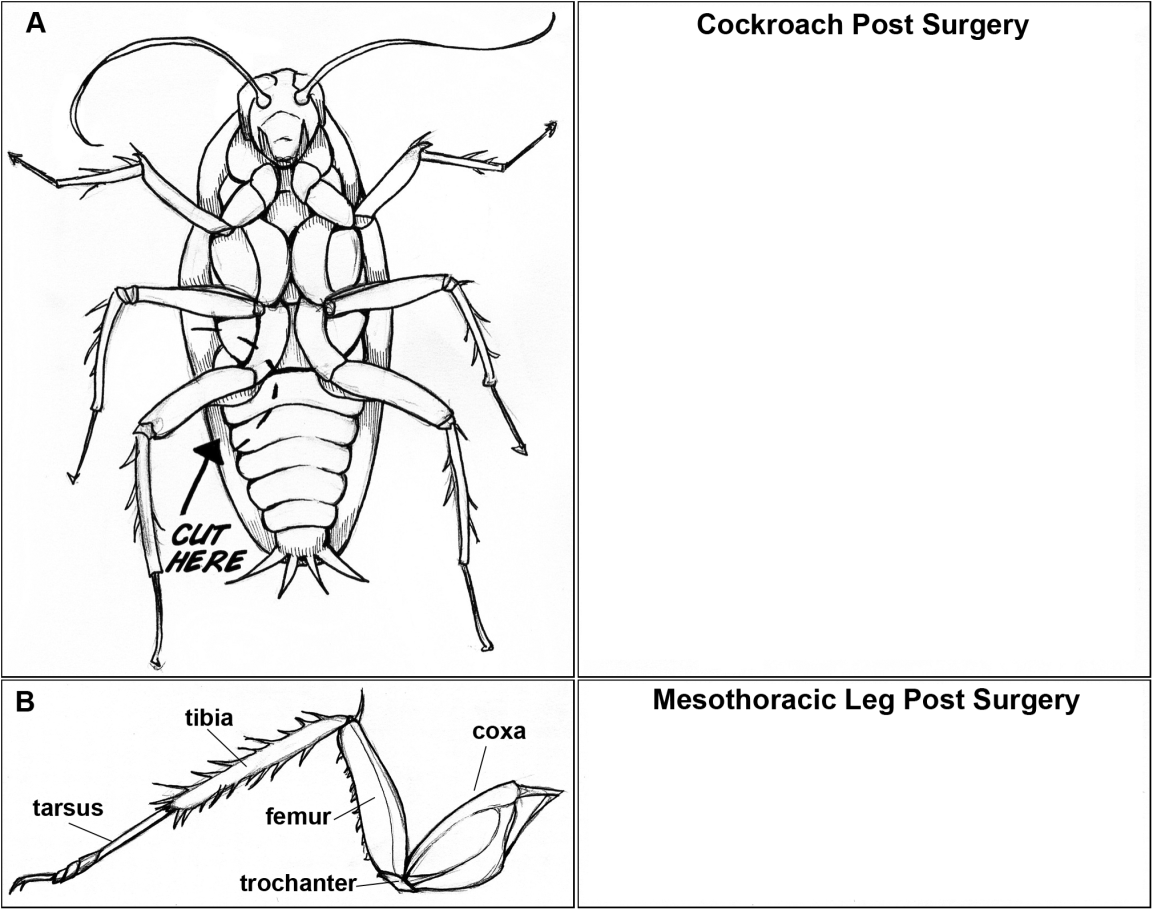
**Exercise 1: Cockroach Mesothoracic Leg Preparation**

This exercise will teach you how to perform a basic experimental setup used to record spikes in a cockroach preparation.

1. Take a cockroach and either put it in ice water or a standard freezer to anaesthetize it. Wait 5-10 minutes, or until the cockroach stops moving. Take care to monitor how long the cockroach has been placed in the water or freezer, as extended exposure to low temperatures can be fatal.

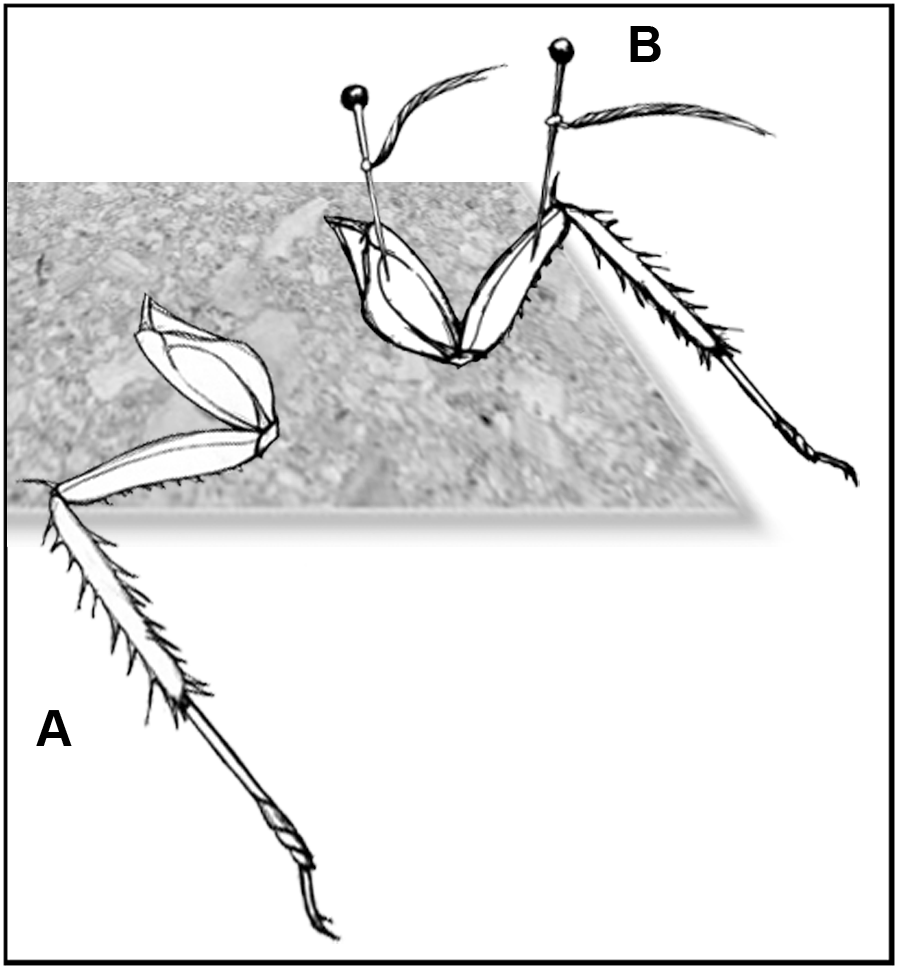
2. Once your cockroach has been anaesthetized, remove it from the water or freezer and place it on your lab bench. Using dissection scissors cut off one of the mesothoracic legs (see Figure 6A below). Make sure you cut close to the thorax (body) so that the coxa remains attached (Fig. 6B).

3. In the space provided below, sketch your cockroach and the leg you have removed. Note any damaged parts if applicable.



5. Return your cockroach to its housing. The leg will grow back if the cockroach is not a full-grown adult yet. One way to distinguish the age of your cockroach is to note whether the wings are fully formed. Adults have wings while younger nymphs do not.

4. Place the leg on the cork found on top of your SpikerBox. Make sure the coxa and femur of the leg are on the cork, while the tibia and tarsus hang freely (Fig 8A). Insert the electrodes through the leg and into the cork. Insert one electrode into the coxa and the second electrode into the femur (Fig 8B). The electrodes will measure action potentials as well as keep your leg in place.



5. Turn your SpikerBox on!

6. If you hear a popcorn sound, congratulations, you have just heard your first neuron firing! If you are not sure whether you are listening to spikes or noise, lightly touch spines located on the tibia with a toothpick. If you don’t hear spikes in response to toothpick stimulations, try reinserting your electrodes, switching which one is in the coxa and femur. Once you are hearing spikes consistently, continue to the next section.

**DISCUSSION QUESTIONS**

**1.** When using a laptop, why do you need to unplug the computer and increase the screen brightness to maximum? What methods do researchers use to decrease electrical interference?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**2.** Why does lowering the temperature of the cockroach make it stop moving? What are other ways you could anaesthetize it?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**3.** Based upon your initial results, are you primarily listening to spikes coming from motor neurons (neurons that tell muscles to contract) or to sensory neurons (neurons that send information from the periphery into the brain). Why?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Exercise 2: How to Record and Analyze Spikes**

In this exercise, you will use several methods to record data from your cockroach leg. Now that you have successfully witnessed spikes you are ready to record, quantify, and graphically present electrophysiology data.

Once your mesothoracic prep is up and running, listen to the spikes coming from the SpikerBox speaker. Can you understand what the neurons are saying to each other? What patterns can you detect while listening?

**Procedure – Initial Setup**

1. Plug the audio adapter cable into the SpikerBox and your computer. Note: for the SpikerBox to record properly, your computer’s audio input jack must be audio only, not a combined audio In/Out jack.

2. Turn on Audacity, open a New window, and ensure the setup described in the previous exercise has been completed.

3. To record from your SpikerBox, simply click the Red Circle at the top of the screen. You can stop recording at any time by pressing the Yellow Square. If you begin to record again, the new “Track” will appear below the previous recordings. You can rename each track by selecting the Audio Track button next to your waveform. If you want to delete a track, select the X button in the upper left of each track.

4. Turn off your cell phone and Wi-Fi. Signal interference from these devices is significant. Ask your teacher permission to send a classmate a text. What does this tell you about how cell phones work?

5. During each experiment, it is wise to record without stopping. Noise created from cell-phones, moving the electrodes, or a variety of other sources can be removed prior to analysis. However, turning your recording on and off may become confusing. The easiest way to keep track of what your data corresponds to is to keep good notes in the space provided.

6. When you save, Audacity will save your “Project” in two forms that may be confusing. The first file saved is a folder ending in “\_data” and the second is a file ending in “.aup.” The .aup file must be in the folder holding the \_data folder. In other words, keep the .aup file in the parent directory of the \_data folder.

**Procedure – Rate Coding**

You can now begin experimenting with your cockroach leg! With your cockroach leg, you will compare how neurons in the leg communicate with no stimulation, a light touch from a toothpick, and a strong blow of air through a straw.

Think about how your brain can tell the difference between a finger lightly touching your arm and a more forceful poke. There are several reasons for why you differentiate the two stimuli. First, the light touch likely stimulates nerves from a very small area while the more forceful poke may stimulate neurons from much more of your arm. Second, the light touch may only stimulate neurons that respond to being compressed. These neurons, among them Merkel cells, send action potentials to the spinal cord and brain in response to light touch. These cells fire faster when stimulated. Therefore a light touch increases their firing rate, which in kind is interpreted by your spinal cord and brain as a light touch. With the more forceful poke, the Merkel cells fire faster, but they may do so much more strongly. Additionally, there may be other neurons stimulated for fire faster that convey another sensation such as pain.

1. Once your cockroach leg is prepared and Audacity is up and running, begin collecting data from your SpikerBox. Ensure your setup is functional by poking the leg a few times. If you see no response, you may need to adjust your electrodes.

2. When you have stable recordings, isolate your leg from any wind and begin the actual recording. Take notes when anything happens to the leg.

3. Record the spontaneous spiking patterns from the leg for 5 minutes. Note the beginning and end time of your control recordings in **Table 1**.

4. Take a toothpick and stimulate the hairs on the tibia of the leg. Try several variations of stimulation including constant pressure or repetitive poking, until you find one that gives you consistent reactions. Write your stimulation method in **Table 1**.

5. Once you have a stimulation method, stimulate the leg repeatedly for 5 minutes. If you need to take a break, do not stop your recording. Take note of the times you are stimulating or resting in **Table 1**.

6. Next, take a drinking straw and blow on the leg. How does the leg react to this stimulation? Try blowing with different amounts of force. Find an amount of force that allows you to maintain a relatively constant flow on the leg.

7. Blow on the cockroach leg for 5 minutes. As with the toothpick, take note of times you need to break, but do not stop your recordings. What happens to the reaction to your blowing over time and after breaks?

**Table 1. Experimental Conditions**

|  |  |  |
| --- | --- | --- |
| **Condition** | **Method Notes** | **Timing Notes** |
| **No Stimulation** |  |  |
| **Toothpick Stimulation** |  |  |
| **Blowing Through Straw** |  |  |

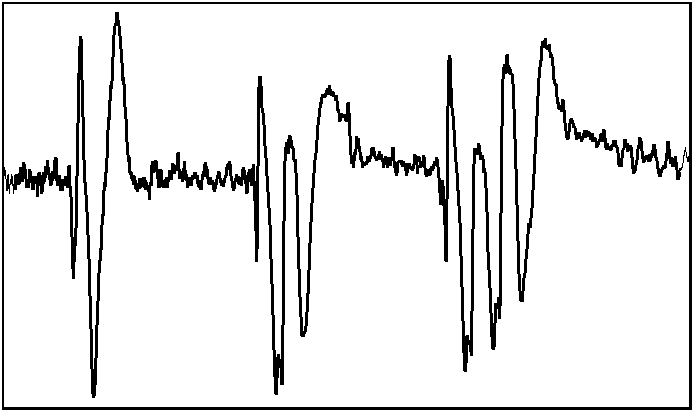
**Basic Analysis of Spikes**

Use the following method to isolate sections of your recordings.

Find a representative section of spikes between 5-10 seconds long and select it with your cursor. Press the left click and drag across the waveform to select. Select a region with clear spikes and as little noise as possible.

Copy the selection (**Control-C**), open a new Audacity window (**Control-N**), and paste the selected 5-10 second waveform (**Control-V**).

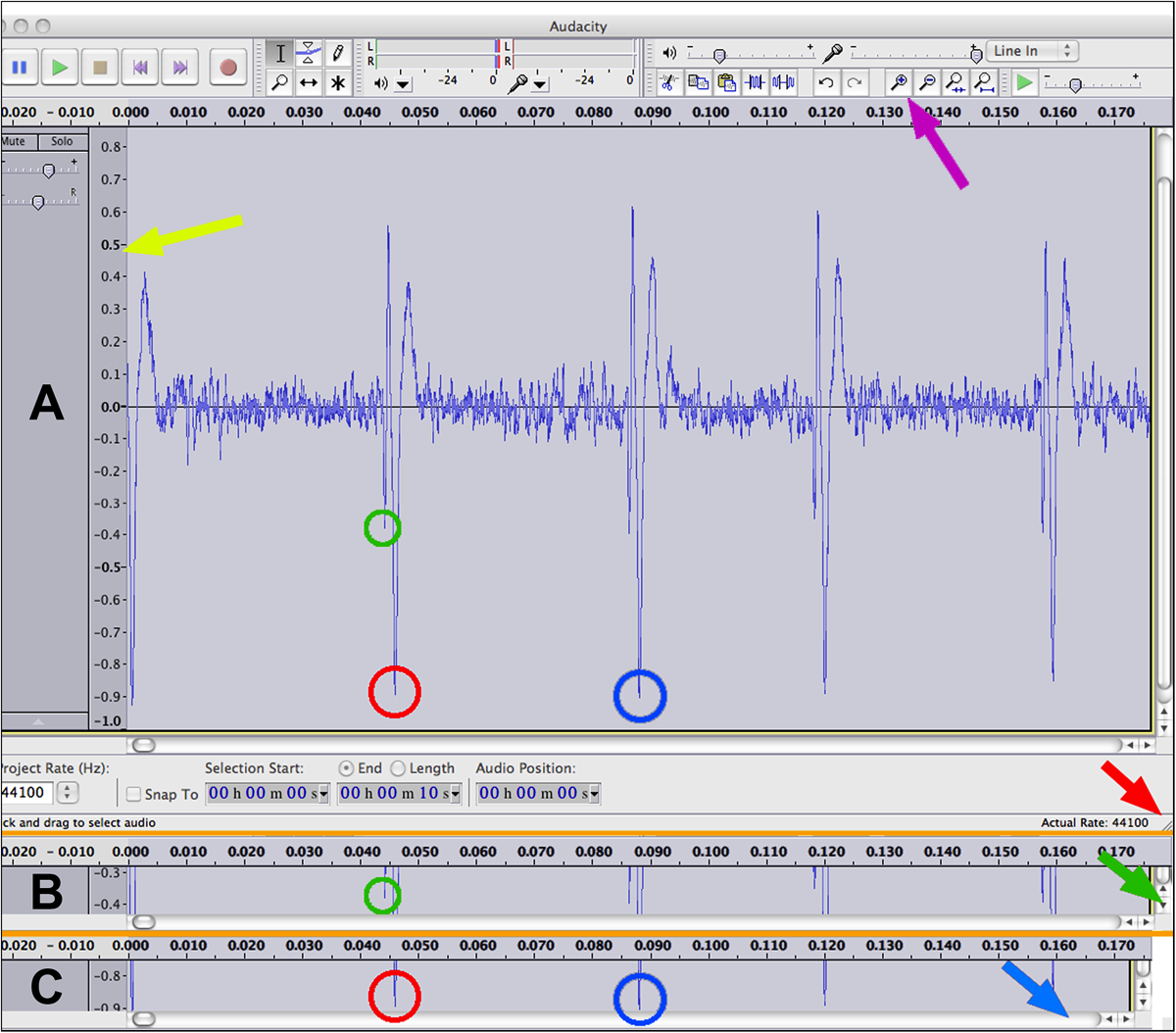
In the **Effect** drop down menu, select **Amplify**. A dialogue box will appear which asks you to select the amount of amplification. The default selection is the amplification that keeps the audio from reaching beyond 1.0 or -1.0 on the scale. Record the **Amount of Amplification** value from the dialogue box in **Table 1**. Use the default settings and click **OK**.

****One of the first things you may see is that spikes do not always occur in a regular fashion.

You may also notice that not all spikes look the same. As shown in Figure 9, spikes may have 2, 3, or even 4 peaks to them. These are not special action potentials, but groups of action potentials.

**Amplitude Analysis - Distribution of Action Potential Amplitudes**

For this exercise, you will be asked to quantify the relative frequency and size of action potentials produced by a cockroach leg in response to your stimulations. Figure 10 provides a screen shot of Audacity that will help you in this process. You will be categorizing the spike peaks by amplitude in a process called **binning**. The bins will be the scale used by Audacity (Yellow Arrow). Keep in mind that you are measuring negative peaks.



Isolate and amplify a 5 second section of recording using the above method. Ensure you have recorded the **Amount of Amplification** in **Table 2**.

Widen your Audacity window to fill the width of your screen using the **Window Resize** tool (Red Arrow).

Use the **Zoom In** tool (Fig. 10A; Purple Arrow) so that you can visualize a small section of your trace, and easily identify the peaks of your spikes. At this point it is easy to eyeball the difference between peaks like those circled in Green and Red. However, for the purposes of quantification, you want to have a consistent methodology that will allow you to reproducibly determine if the peak circled in Blue is different than that in Red.

Look over your data briefly and decide on a threshold for what is noise and you will accept as data. If a peak measuring between -0.2 and -0.3 is sufficiently distinct from the background noise, use this as your minimum negative threshold. Your threshold may be a larger negative number depending on how much noise was recorded. Write the **Threshold Value** in **Table 2**, keeping the number in tenths (i.e. -0.2, -0.3, -0.4).

**Table 2. Amplification and Threshold**

|  |  |  |
| --- | --- | --- |
| **Condition** | **Amplification** | **Threshold** |
| **No Stimulation** |  |  |
| **Toothpick Stimulation** |  |  |
| **Blowing Through Straw** |  |  |

Using the **Window Resize** tool (Red Arrow), shrink the Audacity window vertically, so that the scale on the left of the screen shows only 0.1 of the total waveform (Fig. 10B). This will allow you more accurately determine the bin a peak is in. Some peaks are clearly inside one bin, but some, like the one in the Blue Circle, require a judgment call on your part. Remain consistent in these close calls.

Record in **Table 3**, using hash marks, the number of peaks that end in the bin you have isolated. For example, the peak in the Green Circle has a minimum inside the -0.3 to -0.4 bin, while the peaks found in the Red and Blue Circles do not. Clicking the empty slider at the bottom of the window (Blue Arrow) will move the waveform smoothly, and will allow you to quickly count the peaks that fall within each bin. In many instances, you may have no peaks across an entire waveform inside a bin; this is OK.

Once you have counted all peaks in a bin for the entire 5 second waveform, click the down arrows (Green Arrow) to isolate a new bin. Record the number of peaks in each bin in Table 3.

**Table 3. Spike Amplitudes – No Stimulation**

|  |  |  |
| --- | --- | --- |
| **Bin** | **Peaks Observed** | **Total** |
| **-0.2 to -0.3** |  |  |
| **-0.3 to -0.4** |  |  |
| **-0.4 to -0.5** |  |  |
| **-0.5 to -0.6** |  |  |
| **-0.6 to -0.7** |  |  |
| **-0.7 to -0.8** |  |  |
| **-0.8 to -0.9** |  |  |
| **-0.9 to -1.0** |  |  |

**Table 4. Spike Amplitudes – Toothpick Stimulation**

|  |  |  |
| --- | --- | --- |
| **Bin** | **Peaks Observed** | **Total** |
| **-0.2 to -0.3** |  |  |
| **-0.3 to -0.4** |  |  |
| **-0.4 to -0.5** |  |  |
| **-0.5 to -0.6** |  |  |
| **-0.6 to -0.7** |  |  |
| **-0.7 to -0.8** |  |  |
| **-0.8 to -0.9** |  |  |
| **-0.9 to -1.0** |  |  |

**Table 5. Spike Amplitudes – Blowing Through Straw**

|  |  |  |
| --- | --- | --- |
| **Bin** | **Peaks Observed** | **Total** |
| **-0.2 to -0.3** |  |  |
| **-0.3 to -0.4** |  |  |
| **-0.4 to -0.5** |  |  |
| **-0.5 to -0.6** |  |  |
| **-0.6 to -0.7** |  |  |
| **-0.7 to -0.8** |  |  |
| **-0.8 to -0.9** |  |  |
| **-0.9 to -1.0** |  |  |

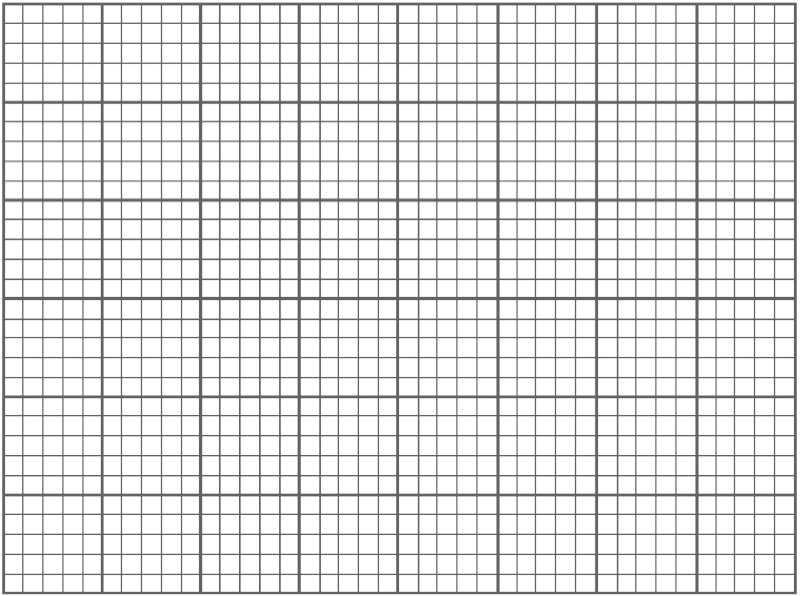
Plot the total number of peaks from each bin on Graph 1.1 for each of your experimental conditions. Draw and label a line for each condition. Make sure you include units and label your axes.

Answer the following:

1. What is the independent variable (x-axis)? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. What is the dependent variable (y-axis)? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Graph 1.1 Title:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_



**DISCUSSION QUESTIONS**

**1.** How do you compare the amplitude values of different peaks once the signal has been amplified?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**2.** How can you tell which electrode is your recording electrode and which is your ground?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**3.** How did the neurons respond to blowing? Was there any sort of attenuation in the neural response over time?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**4.** How many neurons were excited by the toothpick? How many by the straw? How do these responses differ from one another?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_